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(54) Title: NUCLEIC ACID ISOLATION

(57) Abstract

A method for isolating nucleic acids from a biological sample by dissociating protein present in the biological sample, adding a sufficient amount of an inorganic or organic salt to precipitate protein, mixing the biological sample containing the salt for a time sufficient to achieve a satisfactory dispersion of the salt through said biological sample to obtain a protein precipitate, separating the precipitated protein from the biological sample, and separating the nucleic acid remaining in the biological sample. The salt is preferably ammonium acetate and room temperature ethanol is preferably used to precipitate and recover the nucleic acid.

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NUCLEIC ACID ISOLATION

Field of the Invention

The present invention relates to a method for isolating, i.e., separating, nucleic acids from a biological composition. More particularly, this invention relates to the separation of protein from a biological sample containing nucleic acids using salt. The protein is removed prior to the isolation of the nucleic acids.

Description of the Prior Art

The isolation of nucleic acids, i.e., DNA and RNA, from cellular debris is an important and routine procedure in the area of biotechnology. Thus, numerous researchers have attempted to simplify this isolation procedure. If the nucleic acids are purified from complex mixtures of molecules such as cell lysates, generally a proteolytic enzyme such as pronase, proteinase K, chymotrypsin, trypsin, subtilisin, carboxypeptidase and the like or an ionic detergent such as sodium dodecyl sulfate (SDS), sarkosyl and the like is used to dissociate or denature most of the protein which is present. The proteolytic enzymes used are generally selected to be active against a broad spectrum of proteins in order to effectively degrade as much as possible. The protein must be separated or removed from the cell lysate prior to the isolation of the nucleic acids otherwise the protein copurifies along with the nucleic acids. In addition, the protein may interfere with restriction enzymes and with the separation. A poor spectrophotometer reading may also be obtained. The dissociation of protein from the nucleic acid as well as the subsequent removal of

protein is an important step in the isolation of nucleic acids. After degrading the protein, the nucleic acid is typically extracted using an organic solvent.

Marmar, J., "A Procedure for the Isolation of Deoxyribonucleic Acid from Micro-organisms", J. Mol. Biol., Vol. 3, pp. 208-218 (1961) describes a procedure to extract high molecular weight DNA from microorganisms. This procedure is the forerunner of most of today's DNA extraction procedures. Marmar disrupts cells and removes cell debris and protein by organic extraction and centrifugation. RNA is degraded by the action of RNase and is separated from DNA by differential precipitation of DNA using isopropanol. Degration of DNA is prevented by the presence of chelating agents and the ionic detergent sodium dodecyl sulfate.

At the present time, the most common technique used to remove the inactivated or digested protein from a biological composition containing nucleic acids is to use a combination of phenol and chloroform. It is generally believed that two different organic solvents are more efficient than one. The nucleic acid solution is extracted once with phenol, once with a 1:1 mixture of phenol and chloroform, and once with chloroform. A final extraction with chloroform or similar compound removes any lingering traces of phenol from the nucleic acid preparation. This phenol/chloroform extraction procedure is described in Molecular Cloning by Maniatis, T. et al., "A Laboratory Manual", published by Cold Spring Harbor Laboratory, 1982. Maniatis et al defined "chloroform" as a 24:1 (w/v) mixture of chloroform and isoamyl alcohol. "Phenol" is described

as phenol equilibrated with buffer and contains 0.1% hydroxyquinoline and 0.2% β-mercaptoethanol.

Ether is used to remove any residual traces of phenol or chloroform from the DNA solution. Ether, of course, is highly volatile and extremely flammable and should be worked with and stored in an explosion-proof chemical hood.

After inactivating protein present in a biological sample which contains nucleic acids, following current techniques, the aqueous layer containing the nucleic acids in solution is transferred to another tube and the nucleic acids are isolated by precipitating the nucleic acids with about 2 volumes of absolute ethanol. This procedure results in about a 67% ethanol final solution. The precipitated nucleic acids are then recovered by centrifuging or filtering.

A number of disadvantages are associated with this use of phenol and chloroform. Both phenol and chloroform are toxic chemicals which require the use of protective clothing, gloves and fume hoods. Phenol may be absorbed through the skin in sufficient amounts to result in death due to its effects upon the central nervous system as well as damage to the kidneys, liver, pancreas, spleen and lungs. Chronic exposure to low concentrations of phenol vapor may result in digestive disturbances, nervous disorders and dermatitis. Chronic poisoning may be terminal where there has been extensive damage to the kidney or liver. Dermatitis resulting from contact with phenol or phenol-containing products is fairly common in industry.

It is also known that phenol oxidation products can induce DNA damage. This must be taken into consideration for long term DNA storage. Thus it

is important to obtain complete removal of phenol before continuing with the next step in the procedure which is the isolation of DNA.

Chloroform is a suspected carcinogen and has the ability to cause neoplastic growths. Exposure to chloroform vapors can result in conjunctivitis and dilation of the pupils. Prolonged inhalation will cause paralysis accompanied by cardiac respiratory failure and finally death. Exposure to chloroform may also cause profound toxemia and damage to the liver, heart and kidneys.

The phenol and chloroform extraction procedure is also time-consuming and tedious. The necessity of using several re-extraction procedures to remove the phenol and the task of carefully removing the nucleic acid supernatant from the phenol-chloroform interface makes it a lengthy procedure.

Recognizing the disadvantages associated with the foregoing procedure, a variety of other techniques have also been disclosed which either modify the foregoing procedure or use a very different approach.

Gautreau, C. et al, "Comparison of Two Methods of High-Molecular-Weight DNA Isolation from Human Leucocytes," Analytical Biochemistry, Vol. 134, pp. 320-324 (1983) describes two methods of DNA extraction using phenol and chloroform for deproteinization after protein digestion with proteinase K and sodium dodecyl sulfate. One method involves removal of phenol by extensive dialysis with 0.01 M sodium tetraborate and 0.01 M EDTA at pH 9.1. In the second procedure, the DNA was precipitated from the aqueous layer by the addition of 0.1 volume of 20% sodium acetate to the aqueous layer followed by the addition of 2.5 volumes of ethanol. A dilute 1.8%

(w/v) NaCl solution was used to suspend the white cells prior to lysis in each procedure. Both procedures used phenol and chloroform to deproteinize the cell digest.

Longmire, J. L. et al, "A Rapid and Simple Method for the Isolation of High Molecular Weight Cellular and Chromosome-Specific DNA in Solution Without the Use of Organic Solvents," Nucleic Acids Research, Vol. 15, no. 2, p. 859 (1987) describes a method of nontoxic DNA extraction after the digestion of cell lysates with SDS and protease K. The SDS and digestion products are removed by dialysis with 4 x 60 minute washes using 20% polyethylene glycol (PEG). The sample volume decreased and the salts are removed by dialysis with 2 x 30 minute washes with 10 mM Tris-HCl pH 8.0, 1 mM EDTA (TE buffer).

Leadon, S. A. et al, "A Rapid and Mild Procedure for the Isolation of DNA from Mammalian Cells," Analytical Biochemistry, Vol. 20, pp. 282-288 (1982) describes a nontoxic method for the isolation of DNA. Cells are placed on a polycarbonate filter. Cell lysis and digestion with SDS and protease K are carried out on the filter. The solution is allowed to flow through the filter by gravity. The DNA is then removed from the filters by treatment with DNase and 0.5 ml water. The DNA can also be removed from the filter by irradiation with 1000-1500 rad gamma rays followed by an aqueous wash.

Smith, K.C. et al described the use of ammonium sulfate fractionation to separate amino-acid specific RNA. They begin with transfer RNA extracted from guinea pig liver using a phenol procedure to eliminate protein.

Hirt, B., J. Mol. Biol., Vol. 26, pp. 365-369 (1967) describes an extraction method for purifying low

molecular weight nucleic acids such as DNA and RNA away from high molecular weight DNA and proteins. The procedure is performed by resuspending cells in a buffered solution and lysing the cells by the addition of SDS. Cell lysis can be performed at room temperature or 37°C for 10 to 30 minutes. The buffered solution usually contains 10 mM EDTA, pH 8.0 to chelate bivalent cations. An alternative of the lysis procedure resuspends cells directly in 1% SDS, 10 mM EDTA pH 8.0. After cell lysis, NaCl is added to obtain a 1 M final contration. The solution is cooled to 4°C by incubation on ice for 30-60 minutes. At 4°C, high molecular weight DNA, protein and SDS precipitates. After incubation of 4°C, the solution is centrifuged at 4°C for 20-30 minutes at 10,000 rpm to pelletize the precipitate. The supernatant fluid is then decanted. The supernatant fluid contains the bulk of the low molecular weight DNA and RNA from the cells. The processing of the supernatant fluid at this point depends on the desired use of the nucleic acid. Three methods of processing the supernatant fluid that have been used are: 1) addition of 2 to 2.5 volumes of ethanol to the supernatant to precipitate the nucleic acids and recovery of the precipitated nucleic acids by centrifugation; 2) removal of the NaCl and residual SDS by dialysis against a buffer; and 3) extraction of the supernatant fluid using phenol and/or chloroform to remove residual protein and SDS prior to ethanol preciptation or dialysis.

Thus, the prior art has long sought a more efficient, simple and less tedious method to remove protein from a biological sample prior to isolation or separation of nucleic acids. The time involved in the phenol-chloroform separation procedure, the expense

related to waste disposal and the precautions required in handling these two chemicals does not make this procedure well-suited for large-scale mass extraction or for small laboratory use.

Summary of the Invention

An object of the present invention is to develop a fast and efficient method for isolating nucleic acids from a biological sample such as a cell digest or cell lysate.

A further object of the present invention is to provide a method for isolating nucleic acids which does not require the use of hazardous chemicals and maintains a high level of occupational safety for the technician.

An additional object of the present invention is to provide a method for precipitating protein present in a biological sample which uses economical and convenient materials.

The foregoing objectives have been realized by a method for isolating nucleic acids from a biological sample which involves dissociating protein present in a biological sample containing nucleic acids; adding a sufficient amount of organic or inorganic salt to precipitate the protein; mixing the biological sample containing the salt for a time sufficient to achieve a satisfactory dispersion of salt through the biological sample to obtain a protein precipitate; separating the precipitated protein from the biological sample; and finally separating the nucleic acid remaining in the biological sample.

Detailed Description of the Preferred Embodiments

The method of the present invention is suitable for isolating or separating nucleic acids from a biological sample. A biological sample for the purpose of the present invention is defined as any sample or composition which contains nucleic acids. Such samples may include a cell lysate, a cell digest, a sample containing cell leucocytes, a tissue sample, a urine sample, a blood stain or the like. Suitable samples may be obtained from the spleen, kidney, heart, muscle, spermatozoa, intestine, skin, blood and the like from a variety of living creatures whose cells contain nucleic acids including mammalian organisms such as humans. Suitable samples may also include microorganisms such as bacterial cells. Those skilled in the art would recognize suitable biological samples from which nucleic acids such as DNA and RNA may be extracted.

The term, biological sample, is preferably used when describing the present invention since numerous techniques in the area of biotechnology require the isolation of nucleic acids. Typical uses include restriction enzyme digestion, ligation, cloning and the like. The type of sample used for such nucleic acid isolations may vary.

Initially, the proteins including nucleoproteins present in the biological sample are dissociated. Sometimes the term denaturing is used to describe this step. Such techniques are well-known in the art. At the present time the use of proteinase K and SDS is preferred as noted in Molecular Cloning, supra. The product obtained is oftentimes referred to as a cell digest.

Any type of salt may be used in the practice of the present invention. Obviously, the salt must be selected so as not to interfere with the compounds used in any of the other steps during the isolation of the nucleic acids. Suitable salts include all organic salts and all inorganic salts. A representative listing of salts may be obtained by reference to any standard chemistry text. One such text is Lange's Handbook of Chemistry, thirteenth edition, editor John A. Dean, 1985, which is incorporated herein by reference in its entirety.

In its simplest terms a salt can be thought of as an association between a cation and an anion. Suggested anions in the practice of the present invention include but are not limited to chloride, bromide, fluoride, iodide, borate, hypobromite, hypochlorite, nitrate, nitrite, hyponitrite, sulfate, disulfate, sulfite, sulfonate, phosphate, diphosphate, phosphite, phosphonate, diphosphonate, perchlorate, perchlorite, oxalate, malonate, succinate, lactate, carbonate, acetate, benzoate, citrate, tosylate, permanganate, manganate, propanolate, propanoate, ethandioate, butanoate, propoxide, chromate, dichromate, selenate, orthosilicate, metasilicate, pertechnetate, technetate, dimethanolate, dimethoxide, thiocyanate, cyanate, isocyanate, 1,4-cyclohexanedithiolate, oxidobutanoate, 3-sulfidocyclobutane-1-sulfonate, 2-(2-carboxylatoethyl)-cyclohexanecarboxylate, 2-amino-4-(methylthio)-butanoate and the like.

Suggested cations include but are not limited to sodium, potassium, calcium, magnesium, ethylamine, diethylamine, triethylamine, benzylamine, dimethylaniline, N-methylmorpholine, amyloride,

ammonium, lithium, berylium, cesium, zinc, chromium, copper, aluminum, barium, strontium, iron, tin, nickel, barium, silver, platinum, palladium, titanium and the like.

The preferred salt depends on a variety of factors, for instance, if ethanol is used in the final extraction, the salt should preferably be soluble in ethanol so that the residual salt does not precipitate out along with the nucleic acids in the final isolation. Sulfate salts are typically not soluble in ethanol. It would also be advantageous for the salt to be neutral so as not to significantly change the pH or denature the DNA. Further, if SDS is used to digest the protein, it would be preferable if the cation of the salt did not react with anionic SDS which may be present in the nucleic acid digest since it may result in the formation of a sludge which could float on top of the aqueous layer. Under certain conditions and in certain concentrations, heavy metal salts may damage nucleic acids by causing cleavage of phosphodiester bonds. Thus, care must be taken when selecting a heavy metal salt so as not to damage the DNA and RNA present in the biological sample.

As is obvious from the groups of suggested cations and anions presented above, any inorganic or organic salt is suitable to precipitate the protein in the biological sample of the present invention. Preferred inorganic salts include sodium chloride, potassium chloride, magnesium chloride and calcium chloride. Sodium chloride is more preferred. Of the organic salts, ammonium acetate, sodium acetate, potassium acetate and sodium benzoate are preferred. Ammonium acetate is the most preferred salt among both the inorganic and organic salts.

It is believed that the salt dehydrates and thus precipitates cellular proteins which may be present in the biological sample. Thus, it is desirable that the salt have a high solubility in water to allow enough salt molecules to tie up water molecules so that the protein readily dehydrates and precipitates. The amount of salt necessary in the practice of the present invention is an amount sufficient to precipitate protein contained in the biological sample. As noted above, a wide variety of types of biological samples are suitable in the practice of the present invention. And depending on the origin of the sample varying amounts of protein and nucleic acid may be present. Further, some of the biological samples may require a substantial amount of clean-up prior to isolation of the nucleic acids. The amount of salt may range from about 0.01 weight % to about 500 weight % based on the total weight of the biological sample. Preferably, the range is from about 0.1 weight % to about 50 weight %. The salt is preferably added in the form of a saturated solution, however, the salt may be added in solid form or as a dilute solution. For example, in the case of NaCl, a saturated solution is about a 35% (w/v) solution.

After addition of the salt, the biological sample must be mixed in order to obtain a desirable dispersion of salt throughout the biological sample. In most cases, shaking the biological sample containing the salt for approximately fifteen seconds is sufficient. However, depending on the size of the sample, the type of sample, the salt selected, the method of mixing, the temperature of the environment, and like factors, the time for mixing can range anywhere from approximately three seconds to

approximately twenty minutes. The precise mixing time is not critical but rather the biological sample containing salt must be mixed for a sufficient time to achieve a satisfactory dispersion of salt throughout the biological sample and thereby obtain a protein precipitate.

Following the mixing step, the precipitated protein may be separated from the biological sample and the nucleic acid-rich supernatant recovered by any technique known in the art. One such technique is centrifuging the biological sample and the protein precipitate. A single 15 minute spin is generally sufficient and the nucleic acid-rich supernatant may then be readily separated from the precipitated protein. Alternatively, the biological sample containing the precipitated protein may simply be filtered to obtain the nucleic acid-rich liquid.

The nucleic acids in the nucleic acid-rich supernatant may then be separated from the biological composition using any technique known in the art. General methods for precipitating nucleic acid include the use of alcohols, for example, ethanol, isopropanol and the like, or alternatively, polymin-P, streptomycin sulfate, a metal such as copper and the like. This latter technique is particularly useful in the case of high molecular weight nucleic acids.

The most widely used method for concentrating nucleic acids is precipitation with ethanol. The ethanol is preferably at ambient temperature, however, a wide range of temperatures is suitable in the practice of the present invention. The temperature must be sufficient to allow the precipitation of nucleic acid. Obviously, it is also desirable that any other substances also present in the sample not

precipitate out along with the nucleic acid. The precipitate in this widely used method is recovered by centrifugation and redissolved in an appropriate buffer at the desired concentration. This technique is rapid and quantitative even with nanogram amounts of nucleic acid. If the concentration of monovalent cations present in the nucleic acid sample is too high it can be diluted with TE (pH 8.0). If the concentration of salts is too low, an appropriate salt solution may be added. Suggested salt solutions include sodium acetate, sodium chloride and ammonium acetate.

Either DNA and/or RNA may be isolated from the biological sample. RNA requires a slightly higher concentration of ethanol to be precipitated from solution than does DNA. RNA generally requires about 2.5 volumes of ethanol resulting in 71% ethanol final concentration. However, obviously a variety of techniques are known in the art to obtain DNA and/or RNA. A detailed description of methods for the concentration or precipitation of nucleic acids is set forth in Maniatis et al, supra. The entire reference by Maniatis et al is incorporated herein by reference in its entirety.

The isolated DNA and/or RNA can then be used in any standard molecular biological application, including restriction enzyme digestion, ligation and cloning. Additionally, samples can be further processed using DNase and organic extraction to yield RNA suitable for northern blot analysis.

In order to further illustrate the present invention and the advantages thereof, the following specific examples are given, it being understood that

same are intended only as illustrative and in nowise limiting.

EXAMPLE 1

SODIUM CHLORIDE

A tube of anticoagulated blood was centrifuged for approximately 20 minutes at 2000 RPM to separate the cells from the plasma. The white cell (leucocyte) buffy coat was transferred to a 15 ml polypropylene tube. Approximately 9 ml of red cell lysis buffer (0.144 M NH_4Cl, 0.001 M $NaHCO_3$) was added to the polypropylene tube to hemolyze red blood cells. The polypropylene tube was inverted several times and maintained at room temperature for approximately 20 minutes. The tube was spun for 15 minutes at 2000 RPM to separate out the white blood cells. The supernatant was discarded. A white cell pellet remained.

Approximately 3 ml nuclei lysis buffer (10 mM Tris-HCl, 400 mM NaCl and 2 mM Na_2EDTA, pH 8.2) was added to resuspend the white cell pellet. Approximately 0.2 ml 10% sodium dodecyl sulfate (SDS) and 0.5 ml protease K solution (1 mg protease K in 1% sodium dodecyl sulfate (SDS) and 2 mM Na_2EDTA) was added to the white cell suspension to irreversibly inactivate the nucleases and to dissociate the nucleoproteins. The suspension was inverted several times and maintained at 37°C for a minimum of three hours.

Approximately 1 ml of saturated NaCl solution was added to the nucleic acid digest for precipitation and removal of the protein present in the nucleic acid digest. The mixture was shaken vigorously for 15 seconds and centrifuged at 2500 RPM for 15 minutes. The DNA rich supernatant was transferred to a 15 ml

polypropylene tube. Approximately 2 volumes of room temperature ethanol was added to the polypropylene tube to precipitate DNA. This amount of ethanol is insufficient to precipitate the RNA therefore the RNA stayed in solution. The tube was inverted several times until the DNA strands formed and began to stick together. The DNA strands appeared fluffy and white. The DNA was transferred using a plastic spatula to a 1.5 ml microcentrifuge tube containing approximately 100 to 200 microliters of 10 mM Tris-HCl and 0.2 mM Na_2EDTA, pH 7.5 to dissolve the DNA. The DNA was then diluted 1 to 100 in deionized water and quantitated at 260/280 nm (DNA quantity/residual protein quantitation) on a spectrophotometer. Five separate runs were conducted using NaCl and the following 260/280 values were obtained: 1.8, 1.77, 1.9, 1.76 and 1.78. A value of 1.5 or greater is considered desirable.

EXAMPLE 2

POTASSIUM CHLORIDE

Example 2 was conducted in a similar manner as Example 1 except that approximately 1.0 ml of a saturated solution of KCl was added to the cell digest. Fluffy, white strands of DNA were obtained.

EXAMPLE 3

MAGNESIUM CHLORIDE

Example 3 was conducted in a similar manner as Example 1 except that approximately 1.0 ml of a saturated solution of $MgCl_2$ was added to the cell digest. Fluffy, white strands of DNA were obtained.

EXAMPLE 4

SODIUM ACETATE

Example 4 was conducted in a similar manner as Example 1 except that approximately 1.0 ml of a saturated solution of sodium acetate was added to the cell digest. Six separate runs were conducted using sodium acetate and the following 260/280 values were obtained: 1.76, 1.83, 1.8, 1.82, 1.84 and 1.9.

EXAMPLE 5

AMMONIUM ACETATE

Example 5 was conducted in a similar manner as Example 1 except that approximately 1.0 ml of a saturated solution of ammonium acetate was added to the cell digest. The white blood cell sample provided the following 260/280 values: 1.87, 1.84, 1.86, 1.87, 1.87, 1.88, 1.87, 1.86, 1.87, 1.87, 1.88, 1.87, 1.86, 1.84, 1.88 and 1.87.

EXAMPLE 6

AMMONIUM ACETATE

Example 6 was conducted in a similar manner as Example 5 using ammonium acetate, however, the biological sample for Example 6 was derived from tissue. A 260/280 value of 1.77 was obtained.

EXAMPLE 7

RFLP STUDY USING DNA SAMPLE

Example 7 was conducted in a similar manner as Example 1 except that DNA was extracted from anticoagulated blood from five separate individuals. After extraction the samples were quantitated and five ug of each sample was restricted with 8 U/ug of Apa I. DNA was incubated according to manufacturer

specifications (New England Biolabs, Inc.). DNA was electrophoresed in agarose gels, stained with ethidium bromide to check for completeness of restriction and Southern blotted. Membranes were hybridized with human DNA probes specific for detecting Apa I restriction fragment length polymorphisms. Bands were detected with the non-isotopic detection system as described by Dykes et al, "The Use of Biotinylated DNA Probes for Detecting Single Copy Human Restriction Fragment Length Polymorphisms Separated by Electrophoresis", Electrophoresis, Vol. 7, pp. 278-282 (1986). Observed patterns demonstrated no partial restrictions, thus demonstrating that the salting-out extraction procedure did not inhibit the restriction enzymes.

EXAMPLE 8

RFLP STUDY USING DNA SAMPLE

Example 8 was conducted in the same manner as Example 7 except that extracted DNA samples were restricted separately with 6 U/ug of Tag I and 9 U/ug of Msp I. Observed bands after hybridization and non-isotopic detection demonstrated complete restriction.

EXAMPLE 9

RFLP STUDY USING DNA SAMPLE

Example 9 was conducted in the same manner as Example 7 except that extracted DNA samples were restricted with 10 U/ug of Rsa I. Observed bands after hybridization and non-isotopic detection demonstrated complete restriction.

While the invention has been described in terms of various preferred embodiments, those skilled

in the art will appreciate that various modifications, substitutions, omissions, and changes may be made without departing from the spirit thereof. Accordingly, it is intended that the scope of the present invention be limited solely by the scope of the following claims.

WHAT IS CLAIMED IS:

1. A method for isolating nucleic acids from a biological sample, said method comprising:

a) dissociating protein present in a biological sample containing nucleic acids;

b) adding a sufficient amount of organic or inorganic salt to precipitate said protein;

c) mixing said biological sample containing said salt for a time sufficient to achieve a satisfactory dispersion of said salt throughout said biological sample and to obtain a protein precipitate;

d) separating said precipitated protein from said biological sample; and

e) separating said nucleic acid remaining in said biological sample.

2. The method as claimed in claim 1 wherein said protein is dissociated in step a) using a proteolytic enzyme or an ionic detergent.

3. The method as claimed in claim 2 wherein said protein is dissociated in step a) using proteinase K and sodium dodecyl sulfate.

4. The method as claimed in claim 1 wherein said salt is sodium chloride, potassium chloride, magnesium chloride, calcium chloride, ammonium acetate, sodium acetate, potassium acetate or sodium benzoate.

5. The method as claimed in claim 4 wherein said salt is ammonium acetate.

6. The method as claimed in claim 1 wherein said salt is present in the range of about 0.01 wt % to about 500 wt % based upon the total weight of the biological sample.

7. The method as claimed in claim 1 wherein said precipitated protein is separated by centrifuging.

8. The method as claimed in claim 1 wherein said precipitated protein is separated by filtration.

9. The method as claimed in claim 1 wherein the nucleic acid is separated in step e) by the addition of a sufficient amount of alcohol to obtain a nucleic acid precipitate.

10. The method as claimed in claim 9 wherein a sufficient amount of alcohol is added to precipitate primarily DNA.

11. The method as claimed in claim 9 wherein a sufficient amount of alcohol is added to precipitate primarily RNA.

12. A method for isolating DNA from a biological sample, said method comprising:

a) dissociating protein present in a biological sample containing DNA;

b) adding a sufficient amount of a salt selected from the group consisting of sodium chloride, potassium chloride, magnesium chloride, calcium chloride, ammonium acetate, sodium acetate, potassium acetate and sodium benzoate, to precipitate said protein;

c) mixing said biological sample containing said salt for a time sufficient to achieve a satisfactory dispersion of said salt through said biological sample to obtain a protein precipitate;

d) separating said precipitated protein from said biological sample; and

e) separating said DNA remaining in said biological sample.

13. The method as claimed in claim 12 wherein said protein is dissociated in step a) using proteinase K and sodium dodecyl sulfate.

14. The method as claimed in claim 12 wherein said salt is ammonium acetate.

15. The method as claimed in claim 12 wherein the DNA is separated in step e) by the addition of a sufficient amount of alcohol to precipitate DNA.

16. The method as claimed in claim 15 wherein the alcohol is ethanol.

17. The method as claimed in claim 16 wherein the ethanol is added at ambient temperature.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US89/00463

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(4) C07H 15/12; C12P 19/34; C07G 17/00; C07K 1/14, 3/12
U.S. Cl.: 530/344,412 435/91, 267 536/27

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	530/344, 412 435/91, 267 536/27

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

Chemical Abstracts Data Base (CAS) 1967-1989 Keywords: isolation purification/preparation/nucleic acids/RNA/DNA/without or absence phenol or organic solvents/proteins/protein/precipitate

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X Y	Clin. Chem. Vol. 31: 1985, January, Buffone, G.J. "Isolation of DNA from Biological Specemins without Extraction with Phenol". pp. 164-165	1-4,6,7, 9,10,12,13 15-17 5,8,11,14
X Y	Methods in Enzymology, Vol. 65, 1980, April, Davis, R.W., "Rapid DNA Isolations for Enzymatic and Hybridization Analysis". pp. 404-411.	1-4,6,7, 9,10,12,13, 15-17 5,8,11,14
Y	US, A, 4,427,580 (Cornell Research Foundation) 24 January, 1984 See p. 4, line 54 and Figure 1.	8
Y	Scopes, R. Protein Purification 1982 Springer Verlag New York pp. 47-48.	5,14
Y	Maniatis, T. Molecular Cloning, 1982 Cold Spring Harbor, New York, p. 401, 461-462.	11

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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IV. CERTIFICATION

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International Searching Authority	**Signature of Authorized Officer**
ISA/US	Anne R. Brown ANNE BROWN

Form PCT/ISA/210 (second sheet) (Rev.11-87)